MEETING OF THE NORTHWEST SECTION OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY & MEDICINE.

PROGRAM and ABSTRACTS

Lecture Hall 3, Instructional Resources Centre, U.B.C. Saturday, October 27th, 1973

8:45 A.M. Welcome & Opening Statements - Harold C. Copp, Professor & Head, Physiology Dept., Faculty of Medicine

8:50-9:30 A.M. Guest Lecture - Professor John Garrett, King's College, London, England

Dr. Garrett was born in the United Kingdom; he was educated at King's College Hospital where he received his dental and medical degrees. He received a B.Sc. in Physiology from the University of London in 1956 and a Ph.D. in Pathology from London in 1965.

Among his honours he received the Hughes Prize in Anatomy and the Pickering Prize in Biochemistry, both from King's College Hospital in 1955.

From 1961-1964 he held a Nuffield Dental Research Fellowship in the Pathology department and worked with Professor Emmelin at the Institute of Physiology in Lund, Sweden, on salivary gland physiology.

He has held numerous positions at King's College Hospital from 1960 to the present and is currently Professor and Head of the Department of Oral Pathology at that Institution.

He has over sixty publications to his credit in the field of histochemistry, ultrastructure and physiology. His most recent contributions have been in the area of identification of neuro-effector sites in various tissues, and Hirschsprung's Disease. During September and October of this year he is a Visiting Professor in the Faculty of Dentistry, University of British Columbia.

NEURO-EFFECTOR SITES IN SALIVARY GLANDS - J.R. GARRETT

Enormous differences exist in the innervation patterns of salivary glands and it is no longer possible to have a simple unified concept. Two types of neuro-effector sites occur in the glands: (i) "Epithelial", where an axon containing vesicles lies beneath the basement membrane and is in intimate contact with the parenchymal cells; (ii) "Interstitial", where an axon containing vesicles, and running with a Schwann-cell, has a free surface adjacent to a parenchymal cell but lies outside its basement membrane. No obvious pattern exists for the innervation of the various glands in respect to cell type or nerve type. "Epithelial" nerves are more commonly cholinergic but, in certain instances, they are adrenergic. The number of "epithelial" axons decreases with age in some species. Some, but not all, of the morphological findings correlate with known physiological data. The reasons for the complex variations are ill understood but they create a need for awareness of their existence in physiological experimentation.

It is also hoped to mention some preliminary observations (Garrett and Parsons, in press) on permeability of salivary parenchyma to horseradish peroxidase.

SCIENTIFIC SESSIONS

The remainder of the program consists of two simultaneous sessions. Session A consists of papers in the general field of experimental medicine and will be held in Lecture Hall 3 following Dr. Garrett's presentation. Session B, comprising of papers in the general area of experimental biology and comparative physiology, will be held in Lecture Hall 4. Because of the proximity of the two rooms individuals should be able to move freely between sessions.

MEETING OF THE NORTHWEST SECTION OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY & MEDICINE

PROGRAM

SCIENTIFIC SESSIONS - Morning Lecture Hall 3 - Session A
Chairman - J. Tonzetich, H.C. Copp

Lecture Hall 4 - Session B
Chairman - D. Randall, D. Jones

SESSION A - J. Tonzetich, Chairman,
Department of Oral Biology, U.B.C.

9:30-9:45 R. W. Matthews, Department of Anatomy, University of Oregon Dental School

THE MICROVASCULAR ARCHITECTURE OF THE RAT SUBMANDIBULAR GLAND

Few reports have appeared in the literature giving a detailed account of the intra-glandular distribution of blood vessels, and the relationship these have with the secretory and synthetic processes. This study was designed to determine the distribution of blood vessels and capillaries to the component parts of the rat submandibular gland secretory units. Two different techniques were employed; (1) vascular perfusion, and (2) vascular tracing using injected fluorescent dyes. A striking feature of the gland's vascularity was found to be a rich and dense capillary network associated with the walls of the striated and larger ducts. The acini and granular convoluted tubules were invested by a much less dense capillary network. The larger ductal capillary beds were connected to near-by arteries and veins by a plexus of smaller arterioles and venules which themselves showed cross-connections in the form of arterio-venous shunts. Coordination between the onset of secretion and the closure of these arterio-venous shunts is proposed as the mechanism for increasing the blood flow around the secretory units.

9:45-10:00 O.N. Lucas, D. Fujita, Division of Oral Biology, University of Oregon Dental School

HISTOCHEMICAL IDENTIFICATION OF PLASMINOGEN ACTIVATOR IN RAT SALIVARY GLANDS

Plasminogen activator was demonstrated in sublingual, submaxillary and parotid glands. The lytic areas in the fibrin clot induced by activation of plasminogen into the specific fibrinolytic enzyme plasmin were evident microscopically as well defined areas of liquefaction. The fibrinolytic activity was directly related with time of incubation of the tissue with the fibrin clot and found only in the plasminogen-rich fibrin clot. No activity was found in the plasminogen-free fibrin clot. This indicated that the lysis was due to specific activation of plasminogen by the tissue's activator and not due to non-specific proteolysis. The glands' activator was always related with blood vessels, and found to be equally effective in activating human and bovine plasminogen. The activator of plasminogen has been successfully extracted by means of the potassium thyocynate extraction procedure, and its ability to activate plasminogen was measured in Astrup's standard plasminogen-rich fibrin plate.

10:00-10:15 K.H. Haraguchi, V.L. Weimar, Department of Ophthalmology, University of Oregon Medical School

DIFFERENTIAL GROWTH EFFECTS OF MOUSE SUBMAXILLARY GLAND FACTORS ON CORNEAL FIBROCYTES

Five major growth factors have been obtained from mouse submaxillary glands. Four of these represent nerve growth factor (NGF), epithelial-epidermal growth factor (EGF), mesenchymal growth factor (MGF), and thymocyte transforming factor. The fifth factor appears to be a previously undescribed factor promoting powerful growth stimulation of mesenchymal cells. Profound differences were found in the growth stimulating effects of these five different mouse submaxillary gland factors on corneal stromal fibrocytes. Little difference was found between corneal stromal fibrocyte growth in control media and with added NGF or EGF. On the other hand MGF and our previously undescribed growth factor caused profound growth of the corneal fibrocytes. There does not appear to be any correlation between either the esteropeptidase (arginylpeptidase) or general proteolytic activities of the five mouse submaxillary gland growth factors and their growth stimulating activities on the corneal fibrocytes.

10:15-10:30 V.M. Diewert, Department of Orthodontics, Faculty of Dentistry, University of British Columbia

A CEPHALOMETRIC STUDY OF OROFACIAL STRUCTURES DURING SECONDARY CLOSURES IN THE RAT

The relationships between orofacial structures prior to and subsequent to secondary palate closure were evaluated in midsagittal sections of 30 foetal rats. Efforts were made to differentiate between the effects of shelf elevation and the effects of growth of individual orofacial components. In the day before palatal closure, the growth rates (% increase per hour) of the tongue and Meckel's cartilage were greater than those of the mandible and maxilla. The tongue, Meckel's cartilage and the mandible grew in a downward and forward direction similar to the angulation of the incline formed by the nasal septum and medial palatal process of the primary palate. Space for growth of the lower face was provided by maxillary growth, opening of the cranial base, and by extension of the head during the day before palatal closure. Palatal closure took place when the length of Meckel's cartilage exceeded the length of the oronasal cavity measured to the medial palatal process. During palatal closure, the medial palatal process increased in infero-posterior prominence, the cross-section of Meckel's cartilage and the anterior portion of the mandible rotated forward (counter-clockwise, head facing right), and the vertical distance of Meckel's cartilage from the primary palate increased. Anterior tongue protrusion was present during the day of palatal closure but the degree of protrusion relative to the mandible did not change when the shelves elevated. In the day after palatal closure, mandibular growth became more horizontally directed and the rotation of Meckel's cartilage and the anterior portion of the mandible continued. The rate of growth of the mandible was similar to its rate before palatal closure. Growth of the tongue, Meckel's cartilage, the mandible and the medial palatal process appeared to be important factors in creating the environment conducive to secondary palatal closure. Rotation of the anterior portion of the mandible and Meckel's cartilage beginning at the time of palatal closure may produce a change in position of the mid-portion of the mandible, allowing for tongue and palatine shelf exchange.

10:30 - 10:45 Coffee Break

Chairman: L. Kraintz, Department of Oral Biology
Faculty of Dentistry, U.B.C.

10:45-11:00 T.R. Shearer, Department of Preventive Medicine, University of Oregon Dental School

METABOLIC EFFECTS OF SELENIUM IN RELATION TO DENTAL CARIES

Epidemiological studies among children [D.M. Hadjimarkos, in: Advances in Oral Biology, P.H. Staple, Editor, Vol. 3, p. 253,Academic Press, New York (1968); T.G. Ludwig and B.G. Bibby, Caries Res. 3, 32 (1969)], in population groups in the Soviet Union [Suchkov et al., Stomatologia 52(2), 21(1973)], and animal experiments with rats [W. Buttner, J. Dent. Res. 42, 453 (1963)] and monkeys [W. H. Bowen, J. Irish Dent. Ass. 18, 83 (1972)] have demonstrated that somewhat increased intake of dietary selenium during the period of tooth development increases the incidence of dental caries. Three metabolic studies in rats were conducted in our laboratory in order to elucidate how dietary selenium increases the susceptibility of teeth to the carious attack: (1) 3 ppm Se, as Na_2SeO_3, caused significant changes in the pattern of both food and water intake, which can affect the frequency of dental caries, (2) dietary selenium did not significantly change the concentrations of liver or kidney glycolytic and TCA cycle intermediates, and (3) most of the radioactive selenium incorporated into the developing molar teeth was present in the protein fraction of the enamel and dentine. It was concluded that the uptake of selenium into the protein components of the teeth may be involved in the relationship between increased dietary intake of selenium and the increased incidence of dental caries.

11:00-11:15 C. F. Cramer & R. M. Couch, Department of Physiology, Faculty of Medicine, University of British Columbia

ACCUMULATION OF Ca AND P BY GUT EPITHELIAL CELLS AND MITOCHONDRIA

Some properties of accumulation of Ca and P by intestinal epithelial cells and their mitochondria were studied to reveal their role in transmural Ca and P transport. The uptake and release of ^{45}Ca and ^{32}P were measured using isolated cells scraped from the upper half of the rabbit small intestine and incubated in Krebs-Ringer bicarbonate medium at pH 7.4. Mitochondria from the cells were isolated also. The effects of temperature, time, Ca and PO_4 concentration and various inhibitors showed the cells to be a complex system and suggests passive and carrier assisted influx into cells, enhanced by the presence of PO_4 and an active mitochondrial Ca influx. This uptake is opposed by an active cell membrane Ca efflux, and the steady state allows control of transmural Ca transport. The experiments also revealed a possible artifact in some classical in-vitro ^{45}Ca uptake measurements: at Ca concentrations below 4×10^{-4} M, and high Ca binding occurs which may not represent Ca transport into the cell.

11:15-11:30 H. H. Messer & D. H. Copp, Department of Physiology, Faculty of Medicine, University of British Columbia

EFFECTS OF LONG-TERM CALCITONIN ADMINISTRATION TO INTACT RATS

To determine whether animals become refractory to CT administration over a long period, rats received daily CT injections for up to 90 days. The study was divided into two parts: 1) weanling male rats were given 100 mU salmon CT/day for 45 days, followed by 200 mU/day for an additional 45 days; ii) mature (15 week old) male rats received 500 mU CT/day for up to 42 days. Following sacrifice, plasma calcium, phosphorus and acid and alkaline phosphatase levels were measured, and one humerus was taken for Ca and P analyses. Rats receiving

CT for 90 days were significantly ($p < 0.02$) heavier than control (vehicle-injected) rats, showing a growth-stimulating effect of CT of approximately 1% per week. The humerus showed a correspondingly greater dry weight (significance level $p < 0.05$), but there was no difference between control and CT-treated animals in the ash, Ca or P contents of these bones. Plasma phosphatase and Ca levels were unchanged from control values, while plasma P was significantly ($p \leq 0.005$) lower in CT-treated animals. Mature rats given CT for up to 42 days also showed a growth-promoting effect of CT of approximately 1% per week, although the effect was not statistically significant ($0.05 < p < 0.10$). CT continued to exert an effect on plasma CA and P levels throughout the 6 week period. Plasma alkaline phosphatase levels were unchanged by CT administration, while acid phosphatase activity showed a transient decrease which was overcome within 21 days. Thus, there appears to be a variable duration of response to CT, with the most short-lived effect on plasma acid phosphatase, a longer effect on plasma Ca, and no evidence of any loss of response in plasma P and in growth.

11:30-11:45 R. William Clark & E.S. Luschei, Department of Physiology and Regional Primate Research Center, University of Washington

SHORT LATENCY JAW MOVEMENT PRODUCED BY LOW INTENSITY INTRA - CORTICAL MICRO-STIMULATION OF THE PRECENTRAL FACE AREA IN MONKEYS

The face area of the precentral gyrus in monkeys was stimulated intracortically using microelectrodes. Unlike previous face area stimulation studies, the monkeys were awake but mildly tranquilized, the trains of stimuli were of short duration, and the stimulating current was low. EMG activity was recorded from three jaw closing muscles (masseter, temporalis, and medial pterygoid) and one jaw opening muscle (anterior digastric). Jaw movements were recorded using a bi-directional position transducer attached to the ventral tip of the mandible. Short latency jaw movement resulted from stimulation in most locations within the face area. The direction of the evoked jaw movement depended on the location and depth of the electrode. Jaw opening was the most freqeuntly seen type of movement and it was always accompanied by bilateral activation of the anterior digastric. In several instances the activation of the anterior digastric was preceded by a strong, short latency (10-20 msec) inhibition of any ongoing activity in the jaw closer muscles. Jaw closing movements were also seen and were always accompanied by activation of one or more jaw closer muscles. Inhibition of the jaw opener muscles was seen in several instances when jaw closer muscles were activated. Lateral movements of the jaw have also been seen.

11:45-12:00 K. L. Thornburg & J.J. Faber, Department of Physiology, University of Oregon Medical School

AN ELECTRONMICROSCOPIC ANALYSIS OF THE STEADY STATE GRADIENTS OF FERRITIN ACROSS THE NEAR TERM PLACENTA OF THE RABBIT

Solutions of cadmium free ferritin were injected into either the maternal or the fetal blood stream of rabbits of 27-29 days gestation. Sufficient time was allowed after injection for the development of a steady state. Placentas, removed at various postfixation times, were processed for electronmicroscopy after which the ferritin concentration of each placental space was calculated from counts of individual ferritin molecules. When ferritin was injected in the fetal vasculature, the fetal endothelium accounted for 94% of the trans-placental concentration difference with the cytotrophoblast and syncytiotropho-blast accounting for 2.0% and 4.0% respectively. Concentration differences calculated from the counts of maternal ferritin injections were 8.5% and 20.7% and 70.8% for the endothelium, cytotrophoblast, and syncytiotrophoblast, respectively. Discrepancies between concentration drops for each layer after a fetal injection and after a maternal injection can be accounted for if 1)

ferritin is transported actively or if 2) the interstitial spaces in which the counts were made swell during fixation. The latter alternative requires that swelling of the interstitial spaces amounts to 7.0 x in the basement membrane and 3.1 X for the space between the cytotrophoblast and the syncytiotrophoblast.

12:00-12:15 D.A. Lipschitz, J.D. Cook & C.A. Finch, University of Washington School of Medicine

SERUM FERRITIN. ITS MEASUREMENT AND MEANING

Ferritin is a large molecular weight protein with a high iron content. It is found predominantly in the reticulo-endothelial system of the liver, spleen and bone marrow where it functions as one of the 2 major iron storage compounds. Recent studies employing sensitive immunologic techniques have invariably detected ferritin in human serum. A "2-site" immunoradiometric assay has been developed which permits the measurement of minute quantities of ferritin in human serum. Theantigen is insolubilized by means of a ferritin antibody which had previously been adsorbed to the wall of a plastic tube. The test solution is then removed and in a second reaction I^{125}-labeled ferritin antibody is allowed to bind to additional natigenic sites on the insolubilized ferritin molecule. Excess unbound labeled antibody is then removed by washing. The amount of radioactivity remaining on the wall of the tube is proportional to the amount of ferritin initially added. Studies were performed to define the relationship of serum ferritin to iron stores. Normal values were established by performing measurements in male and female subjects between the ages of 20 and 50 years who had a normal hemoglobin level, a transferrin saturation above 20%, and a red cell protoporphyrin less than 100 ug/100 ml RBC. In both sexes values were log-normally distributed with a geometric mean of 94 ng/ml (range 12-437) in 174 males and of 34 ng/ml (range 7-195) in 152 females. This 3-fold difference in means is consistent with prior studies of sex differences in iron stores. Previous studies in normal individuals have shown that both plasma transferrin and iron absorption are inversely proportional to iron stores. A highly significant correlation between the serum ferritin and the TIBC was found in both male ($r=-0.30$, $p<.001$) and female subjects ($r=-.33$, $p=.001$). Further studies in 94 healthy female volunteers demonstrated a high correlation between radioiron absorption and the serum ferritin ($r=0.57$, $p<.001$). In 32 patients with uncomplicated iron deficiency anemia the serum ferritin averaged 3 ng/ml (range 0.5-12 ng/ml). Iron overload resulted in increased levels as high as 15,000 ng/ml. In 20 patients receiving multiple transfusions a direct relationship existed between the ferritin level and the number of transfusions administered. These observations suggest that the serum ferritin provides an accurate estimate of iron stores.

12:15-2:00 Lunch - Faculty Club, Salons C & D

CHAIRMAN - D. H. Copp, Physiology Department, Faculty of Medicine University of British Columbia

2:00-2:15 J.G. Hadley, M.E. Frazier, H. Drucker, and T.K. Andrews, Molecular Biology and Biophysics Section, Biology Department, Battelle, Pacific Northwest Laboratories

USE OF THERMOLYSIN FOR THE DISSOCIATION OF LUNG TISSUE INTO CELLULAR COMPONENTS

A method is described for the dissociation of lung into individual viable cells. Thermolysin was perfused through the pulmonary vasculature and trachea. The lung was then minced and further dissociated by washing with sequential

additions of thermolysin. The results indicate this procedure to be an effective means of dispersing lung tissue into its cellular components. Standard trypsinizing procedures generally yield approximately 5% of the total available lung cells, while the thermolysin treatment increases cell yield to 50% without significantly affecting cell viability.

2:15-2:30 J.C. Hampton, Biology Department, Battelle, Pacific Northwest Laboratories

OBSERVATIONS ON THE PROTECTIVE EFFECT OF WR2721 IN MICE EXPOSED TO X-RAYS

Mice injected intraperitoneally with Compound WR 2721, S-2-(3-Amino-propylamino)ethyl phosphorotioic acid hydrate, within 10 min of exposure to X-rays usually survive 1500R and teated with WR 2721 showed no signs of diarrhea or other symptoms of the G.I. syndrome and no sign of hemorrhage in the viscera at the time of sacrifice. Microscopic examination of the ileum and jejunum revealed that villus denudation did not occur in protected animals at 1500R. Mitotic and labeling indices in treated animals were not significantly different from untreated ones, although these indices were reduced to approximately 50% of control values. Thus it was concluded that WR2721 protects the post-mitotic cell population, rather than the proliferative cells, extending the lifespan of villus cells sufficiently to permit repopulation of the proliferative compartment in the crypts by cells which had survived exposure to X-rays. Apparently a dose of 2500R is sufficient to wipe out the proliferative compartment, at least to the extent that regeneration cannot occur.

2:30-2:45 I.L. Kosin & J.D. Huber, Department of Animal Sciences and Electron Microscope Center, Washington State University

OBSERVATIONS ON THE ULTRASTRUCTURE OF CELLS IN THE BLASTODERMS OF STORED AND NON-STORED CHICKEN EGGS

A standard electron microscope procedure, based on the gluteraldehyde and asmium tetroxide fixation, was used for examining blastodermal cells of white leghorn eggs previously subjected to storage. The storage varied in duration between 0 days and 12 weeks; the ambient temperature in the storage chamber as 12.5°C. Following a prescribed storage treatment and before fixation, some eggs were incubated for 5-16 hours at 39.5°C. Most of the specimens were studied at 4500 X --13000 X, although some electronmicrographs were made at 52000 X. As the storage of eggs increased, marked changes were observed in the cell morphology of affected blastoderms. In particular, this involved the chromosomal complex and the mitochondria. Both showed signs of gradually developing degradation such as the shortening of individual chromosomes and their subsequent clumping into a chromatin "meshwork" or, in the case of mitochondia, the blurring of cristae and their eventual disappearance. The induced process of senescence and moribundity was accompanied by the formation of lipid (?) masses in the cytoplasm and of evidence of physical breakdown in the latter.

2:45-3:00 R.D. Carmichael and J.R. Decker, Jr., Biology Department, Battelle, Pacific Northwest Laboratories

FULGURATION VASECTOMY: AN IMPROVED, PORTABLE INSTRUMENT

Vasectomy has rapidly become an acceptable and common means of male sterilization in many countries. While this operation is ostensibly simple, failure rates (hematoma, sperm granuloma, recanalization) of 2 to 6 percent are reported in the United States. A technique developed by S.S. Schmidt, M.D.,

virtually eliminates failure and complications. This technique, used in over 1,400 vasectomies to date without failure, relies on fulguration of the lumen epithelium and a portion of the muscular wall of the vas deferens using an ordinary unipolar electrocautery instrument. We have developed an instrument specifically designed for the fulguration of the vas deferens using the Schmidth technique but with attributes which make the technique possible under field conditions encountered in lesser developed countries as well as in modern clinics. The instrument has a bipolar, rather than unipolar, electrode which reduced the power demand, increases patient safety and produced a standard lesion by a self-limiting "burn". It is relatively inexpensive, battery powered and, weighing less than 1.5 kg, is quite portable. Successful evaluation of the instrument has been completed on animals and on excised fresh vasa of man. Schmidt is currently conducting a program of clinical evaluation on human patients which will, in the near future, be expanded to include several independent clinics.

3:00-3:30 Coffee Break

Chairman: F.P. Hungate, Biology Dept., Pacific Northwest Laboratories

3:30-3:45 B. Lowell Langille, Zoology Department, University of British Columbia
AN INVESTIGATION OF THE MECHANICAL INTERACTION BETWEEN THE TWO VENTRICLES OF THE MAMMALIAN HEART

The influence of the contraction of one ventricle on pressure generation in the opposite ventricle has been examined in rabbits. When left ventricular pressure was elevated by occlusion of the aortic outflow a marked elevation in right ventricular pressure was observed. However the rate of pressure generation ($\frac{dp}{dt}$) in the right ventricle was independent of the rate of pressure generation in the left ventricle. The occlusion procedure consistently resulted in elevated peak left ventricular ($\frac{dp}{dt}$) whereas peak right ventricular ($\frac{dp}{dt}$) was frequently unaltered. In addition no component of right ventricular ($\frac{dp}{dt}$) could be related to left ventricular ($\frac{dp}{dt}$). Left ventricular pressure was also abruptly elevated by rapid infusion of saline directly into the left ventricle. This procedure, which permitted normal ejection of blood from the ventricle, produced no concurrent rise in right ventricular pressure. Consequently it appears that right ventricular pressure generation depends on the mode of contraction of the left ventricle rather than on the actual left ventricular pressure produced. It has yet to be established that the mechanical coupling observed during isovolumic left ventricular contraction is of physiological importance. When right ventricular contraction is of physiological importance. When right ventricular pressure was elevated by occlusion of the pulmonary outflow a parallel rise in left ventricular pressure was produced. The mechanism for this right to left ventricular coupling has not yet been investigated although an analysis in terms of a simple application of Laplace's law appears possible.

3:45-4:00 Olav M. Sola, Stevens Memorial Hospital, Edmonds, Washington
STRETCH-INDUCED HYPERTROPHY OF FIBERS OF THE POSTERIOR LATISSIMUS DORSI OF THE ADULT CHICKEN AND A REVIEW OF STRETCH-INDUCED HYPERTROPHY

Our recent studies have verified our earlier observations that when sufficient hypertrophy is induced, with or without denervation, hyperplasia of the slow-twitch, intermediate fibers occurs in the Anterior Latissimus Dorsi of white leghorn chickens. Hypertrophy of the fast-twitch red fibers has been produced

with stretch; however, no incidence of hyperplasia has been found. The purpose of this investigation is to determine if hypertrophy can be produced in white muscle fibers and to observe if hyperplasia can occur in either white or red muscle fibers subjected to stretch. The origin of the Anterior Latissimus Dorsi muscle of the white leghorn chicken was divided allowing the muscle to retract laterally. Weights of either 100 or 200 grams were attached to the wing and the muscle removed, weighed and examined histo-chemically at serial intervals. Weight increase of 100% was noted with hypertrophy of both red and white fibers resulting. Microscopic sections revealed both fibers nearly doubling in diameter. No evidence of hyperplasia such as occurred in the slow-twitch intermediate fibers was found. A marked tendency for connective tissue of the Anterior Latissimus Dorsi muscle to bridge the space and connect to the spinous processes was noted. Even with the muscle being severed, the Anterior Latissimus Dorsi with added tension showed hypertrophy. The study appears to strengthen the hypothesis that only stretched, slow-twitch intermediate fibers respond with hyperplasia as well as hypertrophy.

4:00-4:15 C. Slonecker, Department of Anatomy, Faculty of Medicine & C.P. Osmanski, Department of Oral Biology, Faculty of Dentistry, University of B.C.

ACUTE INFLAMMATORY REACTIONS IN PROTEIN-DEFICIENT RATS

The effects of a low protein dietary intake on the formation of an acute inflammatory exudate in rats were studied. Male weanling rats were maintained for 4 weeks on a diet containing 0.5% protein while controls were given a diet containing 18.0% protein. An acute peritonitis was induced by an intraperitoneal injection of a suspension of 1% latex particles (0.794 dia) in sterile Ringer's solution. The cellular composition of the inflammatory exudate was then examined on smear preparations at 0, 4, 8, 16, 24, 36, 48 and 60 hours. Marked differences were noted in the peritoneal exudate from the rats which had been maintained on low protein diets. A striking decline in the number of mononuclear macrophages was evident and maximal influx of macrophages into the exudate occurs at a later stage. Eosinophils were greatly increased between 24 and 48 hours in the exudates of the experimental animals. A significant lymphocytic response was also noted between 24 and 48 hours. The lymphocytic population demonstrated a greater proportion of larger sized cells and most showed greater quantities of basophilic cytoplasm. This cellular composition suggests that an allergic and/or immunological response could be occurring in the exudate of the experimental animals.

Note: Business Meeting will be held after lunch in the Faculty Club

MEETING OF THE NORTHWEST SECTION OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY & MEDICINE

SCIENTIFIC SESSION B

Lecture Hall 4, I.R.C. Building

Chairman: Dr. D. Randall,
Zoology Department,
U.B.C.

9:30 - 9:45 Helen E. Gruber, Department of General Science, Oregon State University

Growth: 12 hr. light period, 12 " dark "

Light: Photosyn. Dark:

VARIATION IN EYESPOT ULTRASTRUCTURE IN CHLAMYDOMONAS REINHARDI (ac-31)

The eyespot is a conspicuous feature of Chlamydomonas reinhardi, and its intracellular position is constant, being situated midway between the anterior and posterior ends of the cell. This organelle is considered to function as either a primary photoreceptor or a shade over some other photosensitive region of the cell. In either case, one would expect the fine structure to be quite uniform from one cell to another. The observations reported here document several variations in morphology, consisting of irregular curved bodies associated with the eyespot region, distended eyespot complexes accompanied by displaced and possibly disrupted chloroplast and cell membranes and crystalline arrays of dense bodies. Microtubules in the eyespot region appeared to bear a normal relationship to the eyespot, but since microtubular connections between eyespots and flagella were not found, this study neither supports nor refutes the theory that transfer of information between eyespot and flagella is mediated by microtubules. Two variations, crystalline arrays of dense bodies and curved, rod-like bodies, appear to be related and an hypothesis for the transformation from one to the other will be presented and discussed.

9:45 - 10:00 Bela Sivak, Department of Botany, University of British Columbia
EFFECTS OF FUNGAL INFECTION ON WOUND HEALING IN POTATOES

The initial stages of wound healing was studied in potato tuber slices, and also the manner in which unspecialized (Phytophthora erythroseptica) and highly specialized (P. infestans) fungal pathogens of potato might affect this process in terms of RNA levels and species of RNA synthesized. RNA was isolated from control and inoculated tuber slices (diameter 24 mm; thickness 3.0 mm) at 5h and 10h after inoculation (or slicing) following a 2h-long feeding with carrier free phosporic acid ^{32}P. In each RNA sample the high- and low-molecular wt RNA were separated by precipitation with salt (1.4 M NaCl) and 2-ethoxy ethanol respectively. Specific activities and base composition of both salt- and 2-ethoxy ethanol-precipitated fractions of each RNA sample were then determined. In uninoculated slices there was a marked increase in both high- and low-molecular weight RNA from 5 to 10h after slicing. In addition, there was an AMP-rich fraction which precipitated with the high molecular wt RNA at 5h after slicing. At 10h after slicing, however, the AMP-rich RNA was confined to the low-molecular wt RNA fraction. In tuber slices inoculated with P. infestans, RNA levels did not increase as markedly as in the uninoculated slices. Moreover, the synthesis of AMP-rich RNA was maintained only when the inoculum of this fungus was separated from the tuber tissue with

EFFECTS OF FUNGAL INFECTION ON WOUND HEALING IN POTATOES (Cont'd)

dialysis membrane, the AMP-rich fraction was preserved. In tissue slices infected with mycelial inoculum of P. erythroseptica the high-molecular wt RNA increased significantly from 5 to 10h after inoculation. However, the increase of low-molecular wt RNA was not as significant. AMP-rich RNA was detected only at 5h after inoculation in the fraction precipitated with salt. At 10h after inoculation, whether the inoculum was applied directly or separated by dialysis membrane, from the tuber slices, AMP-rich RNA could not be detected in either RNA fraction.

10:00 - 10:15 John S. Edwards, Department of Zoology, University of Washington
USE OF ULTRAVIOLET MICROBEAM FOR CAUTERY OF INSECT EMBRYOS

accurate regeneration of nerve stimulus

Neural regeneration in the abdominal cerci of the house cricket, Acheta domesticus provides a relatively simple system in which to study specificity and plasticity in the nervous system. In order to prepare animals in which the ganglion under study does not receive its normal input of sensory fibres from the periphery, it is necessary to remove the rudimentary appendages from the embryo before sensory differention takes place. This was achieved by irradiating the cercal rudiment in an untraviolet microbeam. Animals so treated developed normally and hatched without abdominal cerci. It is proposed to use such animals later in post-embryonic development as recepients of grafted regenerate cerci. While the normal animal regenerates cerci vigorously during postembryonic development, cautery of the embryonic rudiment does not give rise to a regenerate. The significance of these observations will be discussed.

10:15 - 10:30 A.W. Martin, Department of Zoology, University of Washington
THE 'PROTECTIVE-SYNTHESIS' OF HIPPURIC ACID IN A CEPHALOPOD MOLLUSC

Since the identification of hippuric acid in 1830 by Justus Liebig there has accumulated a vast literature on the "detoxication" of benzoic acid, or the "protective-synthesis" of hippuric acid. In our chemically enlightened day the forms of protective-synthesis employed by one animal species have been shown to be numerous and to demand simultaneous assessment of several products of excretion. But this sophistication is restricted to relatively few species, and they are either mammals or birds. In the Phylum Mollusca almost nothing is known of this whole field; and in the Class Insecta of the Arthropoda the analysis is beginning with a study of the very complicated organic chemicals used as insecticides. This report will deal with a determination of the basic facts of the single product hippuric acid, by the large local octopus: Octopus dofleini martini.

10:30 - 10:45 Coffee Break

Chairman: A. W. Martin, Department of Zoology, University of Washington

10:45 - 11:00 William K. Milsom (Introduced by A.W. Martin), Department of Zoology, University of Washington
BUOYANCY CONTROL IN TURTLES (CARETTA CARETTA (LINNÉ) AND CHRYSEMYS PICTA)

All aquatic turtles regulate their buoyance state continuously during diving and swimming by regulating lung volume. Such specific gravity adjustments may be small immediate adjustments for diving or long term adjustments for large changes in body weight requiring substantial changes in lung volume. The inflexible shell of freshwater turtles necessitates some compensating mechanism for internal (lung) volume changes of large magnitude. The reduced flexible shell and enlarged cervico-axillary and inguinal regions of marine turtles enable body volume alterations to compensate large lung volume alterations. Immediate buoyance compensation (small lung volume alterations) for diving is achieved by this latter method in both turtles studied. Smooth muscle is found in varying amounts in the lungs of all aquatic turtles studied. These muscles do not participate in ventilation but contract prior to closure of the glottis at the termination of each breath. The degree of contraction determines the lung volume during the ensuing apneic period. On the last breath prior to a dive, lung volume is reduced (specific gravity increased) and the turtle descends (actively or passively). Air is not released during the dive. A larger lung volume is re-established on the first post-dive breath restoring positive buoyancy.

11:00 - 11:15 N.H. West, Department of Zoology, University of British Columbia
THE MECHANISM OF VENTILATION AND THE MECHANICAL WORK OF BREATHING IN RANA PIPIENS

The mechanism of ventilation in the frog Rana pipiens was studied by recording pressure and volume changes from the buccal cavity and lungs, together with muscular activity from the nares and glottis. Two distinct types of ventilation cycle occurred; buccal ventilation cycles in which the glottis remained closed and the nares open, and lung ventilation cycles which involved the active participation of the nares and glottis. Lung ventilation cycles occurred in regular sequences in which each cycle reached a peak pressure 10-20% higher than the preceding cycle until the lungs were fully inflated. These sequences were separated by periods during which the inflated lungs were isolated from the buccal pump with the glottis closed. The recording of pressure-volume loops from the buccal pump enabled an analysis to be made of the proportion of work available for lung inflation after losses against viscous and flow-resistive forces in the pump itself. By recording representative sequences of work loops from the pump it was also possible to measure the mechanical work output of ventilation in restrained but unanaesthetised frogs. This proved to be in the range of 0.5 joules per hour for frogs with a mean weight of 64 grams. By combining mechanical work rate with a value for mechanical efficiency, obtained by estimating the maintenance heat and heat of shortening of the respiratory muscles, it was possible to arrive at a value for the oxygen cost of ventilation in Rana pipiens. This value was between 2 and 7%.

11:15 - 11:30 O.S. Bamford & David R. Jones, Zoology Department, University of British Columbia
THE EFFECT OF GLOTTAL RECEPTORS ON THE INITIATION OF APNOEA AND BRADYCARDIA DURING DIVING

It has been shown that stimulation of the glottal branch of the 9th cranial nerve can induce apnoea and profound bradycardia and we have considered the possibility that, under natural conditions, stimulation of glottal receptors by water contributes to reflex inhibition of respiration initiated by receptors in the nares. Nerve recordings made from small numbers of units in the glottal nerve revealed that some receptors responded to all aqueous liquids (e.g. water, saline, sea-water, acids, alkalies) but not to low viscosity oils. These 'water receptors' showed a marked temperature sensitivity being completely inhibited at water temperatures above 40° C. That these receptors are involved in the generation of apnoea was confirmed in the whole animal by passing various solutions over the glottis. All aqueous solutions, except those at 40° C, caused maintained apnoea whereas oils caused little or no change in the breathing pattern. Receptors innervated by the 5th nerve were less specific than the glottal receptors in that maintained apnoea could occur with warm water and there were even brief periods of apnoea with oil. Regardless of the stimulus used to induce apnoea, no significant differences occurred in development of diving bradycardia.

11:30 - 11:45 David R. Jones, Zoology Department, University of British Columbia
THE OXYGEN COST TO THE METABOLISM AND EFFICIENCY OF BREATHING MOVEMENTS IN TROUT. (SALMO GAIRDNERI)

Fish were placed in a ventilation chamber which allowed separation of inspired and expired water by means of rubber membrane sewn around the mouth. Since trout cease breathing movements when on an adequate level of forced ventilation, and the apparatus allowed independent control of the water levels in the inspired and expired water compartments of the ventilation chamber, it was possible to measure $\dot{V}O_2$ under conditions of forced, normal and loaded ventilation. Compared with the situation when the fish was breathing normally $\dot{V}O_2$ fell by 9.71 $\pm$ 1.86% when on forced ventilation and increased when pumping against a water head of 5 mm H_2O by 8.05 $\pm$ 2.13%. A large load (head of 10-15 mm H_2O) was only tolerated by a few fish and $\dot{V}O_2$ increased by 17.99 $\pm$ 16.18% compared with normal ventilation. Data from fish whose $\dot{V}_G$ under forced or loaded conditions, varied by more than $\pm$20% of normal $\dot{V}_G$ were not included in this analysis. The work done (mean pressure x $\dot{V}_G$) during breathing was measured in fish with buccal and opercular cannulae. Under conditions of both normal and loaded ventilation the opercular expiration phase dominated in its contribution to the total mean pressure generated during each breathing cycle. The efficiency of normal ventilation was low but increased to 1.7 $\pm$ 0.6% when power output went up by 52%. The results are discussed in the light of determinations by others and the contribution of kinetic, inertial and negative work components to the work of breathing are examined.

11:45 - 12:00 Miriam McLean (Introduced by A.W. Martin) Dept. of Zoology, University of Washington, Seattle, Washington
AXONAL REACTIONS IN CRICKET INTERNEURONS AFTER LESIONS OF THE VENTRAL NERVE CORD

The insect nerve cord essentially consists of a median ventral chain of bilaterally-symmetrical ganglia interconnected by paired bundles of nerve fibers. Unilateral experimental lesions of the interganglionic connectives are generally well-tolerated. This sort of operation provides a convenient preparation for studying the reactive changes of interneurons over a period of time. One can follow degenerative or regenerative responses of functionally or morphologically identified cells. I have made surgical lesions of cricket connectives at various stages of postembryonic development. I shall describe some short-term ultrastructural changes in the axons of giant interneurons whose cell bodies lie in the terminal ganglion. Responses to such operations appear to be more extreme in the segment of the axon proximal to the wound than in the distal axon segment. Contrasts in axonal reactions between mammals and insects will be discussed.

12:00 - 12:15 D. F. Stiffler & R. H. Alvarado, Dept. of Physiology, University of Oregon Medical School and Dept. of Zoology, Oregon State University
RENAL RESPONSE TO REDUCED OSMOTIC LOAD IN LARVAL SALAMANDERS

Renal function was studied in larval Ambystoma gracile and Dicamptodon ensatus exposed to several osmolalities. Clearance methods were employed in free-swimming larvae using inulin as a GFR marker. GFR, as calculated from the rate of appearance of inulin in the bath, declined from 0.113 to 0.023 ml/10g-hr. (A. gracile) and from 0.166 to 0.006 ml/10g-hr. (D. ensatus) between 2 mOsm (tap water) and 300 mOsm (sucrose). In experiments involving urine collection (A. gracile), urine flow decreased from 0.078 in controls to 0.018 ml/10g-hr. in the 200 mOsm group while GFR decreased from 0.104 to 0.028 ml/10g-hr. The latency for the reduction in GFR is about two hours; however, the return to normal GFR after return to tap water takes about six hours. Fractional reabsorption of water increased from 25 to 37 per cent in the 200 mOsm group while fractional reabsorption of Na, K and Cl decreased slightly. The results indicate that both glomerular and tubular factors are involved in the response to reduction in osmotic load; however, the former predominate.

12:15 - 2:00 Lunch - Faculty Club, Salons C & D

Chairman - David Jones

2:00 - 2:15 Roy R. Adee, Biology Department, Battelle, Pacific Northwest Laboratories
TEMPERATURE DEPENDENT VARIATIONS IN MEMBRANE SYNTHESIS IN THE EYE OF THE RAINBOW TROUT

The potential use of Columbia river water as a coolant for future nuclear development has generated considerable interest in the physiological changes that occur in rainbow trout with increased river temperature. The rods from the retina of the trout eye were used as a model to study the rate of membrane synthesis, since membranes comprising the outer segment are assembled at the isthmus between inner and outer segments and then migrate to the tip of the outer segment. The experimental protocol is as follows: Rainbow trout that were thermally adapted at two temperatures, 8°C and 18°C were injected I.V. with about

TEMPERATURE DEPENDENT VARIATIONS IN MEMBRANE SYNTHESIS IN THE EYE OF THE RAINBOW TROUT (Cont'd)

0 μCi of a mixture of 4 tritiated amino acids. Serial sacrifices were made every week with the 18°C adapted fish and every 2 weeks with the 8°C adapted fish. The retinas were processed for E.M. examination and embedded in epoxy resin. Sections about 1 μm thick were mounted on glass slides, coated with autoradiographic emulsion and exposed for about 90 days. The sections were then examined with a Zeiss photomicroscope and measurements taken of the exposed silver grains on the rods of the fish retina. The results indicated about a three-fold increase in the rate of membrane synthesis for the trout adapted at 18°C compared to those adapted at 8°C.

2:15 - 2:30 F.D. Klopfer & G.L. Goodrich, Washington State University
(Introduced by Igor L. Kosin)
ACCOMODATION FOR AIR VISION IN THE DOGFISH AND RATFISH

Some elasmobranch and chimeroid species put their heads out of water to observe objects presented above the water surface. For vision both below and above water, accomodation must compensate for the power of the cornea in air as compared with water, and this is a function of the corneal curvature. We have measured the curvatures in dogfish (Squalus suckleyi) and ratfish (Hydrolagus colliei), both of which view objects above water, and hammerhead sharks (Sphyrna lewini) which does not. Assuming the usual refractive indices for aqueous, lens and vitreous, these animals would be nearly emmetropic at 3 meters underwater, but would be about 20 diopters myopic in air. Several subsidiary mechanisms that could reduce the magnitude of myopia were considered. First, photographs using a slit lamp with ratfish and dogfish indicate that the anterior chamber effectively disappears when the eye is in air, and this decreases the power of the eye. Secondly, relaxing the dogfish extraocular muscles with MS-222 or with d-tubocurarine chloride reduces the power of the cornea in both the horizontal and verticle axes by about 12%. Thirdly, observation and photographs of dogfish and ratfish with their eyes out of water as they "observe" objects above water indicate that peripheral vision, which involves a shorter image distance, is a likely technique for reducing the myopia. The hammerheads were never observed to put their eyes out of water in visual exploration; their anterior chambers were deeper and visible in both air and water, and neither curare nor death significantly altered their corneal curvatures.

2:30 - 2:45 Aubrey Gorbman, Department of Zoology, University of Washington
IMMUNOLOGICAL CROSS-REACTIVITY BETWEEN PURIFIED THRYOGLOBULINS OF MAMMALS, REPTILES AND BIRDS

No abstract received.

2:45 - 3:00 Sema Ergezen, Department of Zoology, University of Washington
REGIONAL DIFFERENCES IN RNA SYNTHESIS IN BRAINS OF FROGS

No abstract received.

3:00 - 3:30 Coffee Break

Chairman: A. Gorbman, Dept. of Zoology, University of Washington

3:30 - 3:45 Khay Huat Khoo, Department of Zoology, University of British Columbia
THE ROLE OF GONADAL STEROIDS IN THE ENDOCRINE CONTROL OF VITELLOGENESIS IN GOLDFISH (CARASSIUS AURATUS)

In studies of the pituitary-gonadal relations, it is evident that the pituitary regulates both oogenesis and steroidogenesis. In hypophysectomized teleosts both vitellogenesis and steroidogenesis are inhibited. The possibility that pituitary control of vitellogenesis could be mediated by gonadal steroids was studied. Female goldfish were hypophysectomized and kept in saline for 5-6 weeks. This was to ensure that all yolky oocytes were completely reabsorbed. An aqueous suspension of 10 µg./g. body weight, of steroids was injected into the peritoneum of the hypophysectomized goldfish. Steroids known to occur in the metabolic pathway of steroidogenesis in teleost were studied. The steroids were injected once every third day for one month. Of the various steroids examined, the estrogens (estradiol-17β, estrone and estriol) induced the formation of yolk vesicles in the oocytes of hypophysectomized goldfish. Pregnenolone, on the other hand, induced the formation of only the second type of yolk inclusions, the yolk granules. The other steroids had no apparent effect on vitellogenesis. The possible role of steroids in vitellogenesis of teleosts is discussed.

3:45 - 4:00 Deborah L. Christensen
THE EFFECT OF STRETCH ON INNERVATED OR DENERVATED ADULT CHICKEN SLOW MUSCLE AS SHOWN BY WEIGHT GAIN AND HISTOLOGICAL CHANGES

White Leghorn roosters over one year of age and averaging 2000 grams each in body weight were provided with weights of 100 and 200 grams which were attached to their left fore-wings near the elbow, applying moderate or extensive stretch to the anterior latissimus dorsi muscle. Right wings were used as controls. Gain in wet weight of the entire left muscle was correlated with the histological changes observed from myosin ATPase and other histochemical preparations of cross-sections taken from a specific area of the muscle. Comparisons were made between muscles that had been: denervated only; weighted with 100 grams for various lengths of time with innervation left intact or denervated; weighted with 200 grams with innervation left intact or denervated. Results indicated that: 1) Histological changes kept pace with the rate of the hypertrophy and followed set patterns; 2) the rate of hypertrophy was directly dependent on the amount of stretch applied to the muscle, although modified by denervation; 3) hypertrophy occurred whenever weight was applied to the wing and endured as long as the muscle was kept under tension; and 4) hyperplasia occurred when the hypertrophy exceeded 70% within 1 to 2 weeks. Fiber counts of entire muscle cross-sections were taken from myosin ATPase preparations. Fragments split from existing fibers, thereby staining similarly, were excluded, but new fibers, which stained black and corresponded in other characteristics to embryonic muscle fibers, were included in the counts. The total number of fibers per cross-section increased by an average of 70 fibers per week in the left muscle while the control remained relatively the same, accumulating in 8 weeks of extensive stretch over 500 more fibers on the left than the right.

4:00 - 4:15 S.S. Duffey & G.H.N. Towers, Department of Botany, University of British Columbia.
DEFENSIVE SECRETIONS: THE BIOSYNTHESIS OF HYDROGEN CYANIDE IN MILLIPEDES

No abstract received.

Note: Business Meeting will be held after lunch in the Faculty Club

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